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ASSOCIATION OF *PARATRECHINA ARENIVAGA* (HYMENOPTERA: FORMICIDAE), WITH NYMPHS OF *OECLEUS BOREALIS* (HOMOPTERA: CIXIIDAE)[1,2]

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Abstract.—The previously unknown nymphs of the planthopper *Oecleus borealis* Van Duzee were found in the subterranean nests of the ant *Paratrechina arenivaga* (Wheeler). The nymphs appear to associate only with this ant species. Food plants of the *O. borealis* nymphs are suspected to be the roots of sand pine *Pinus clausa* (Engelm.) Sarg., saw palmetto *Serenoa repens* (Bartr.) Small, or turkey oak *Quercus laevis* Walter, although radioisotope experiments to establish this were inconclusive.

The family Cixiidae is a member of the superfamily Fulgoroidea, or planthoppers. This family, to which *Oecleus* belongs, has an extensive distribution in the temperate regions of the world (Van Duzee, 1908). J. P. Kramer (1977) listed 40 species of *Oecleus* in the U.S. The distribution of the genus is mostly western, with only 3 species occurring east of the Mississippi River. *O. borealis* occurs from the Gulf states north to New York and west and north to Texas and Kansas (Kramer, 1977). Literature on cixiid biology is extremely sparse and is contained for the most part, in small notes following taxonomic descriptions. Dozier (1926) took both adult sexes of *O. borealis* by sweeping grasses in pinewoods. He speculated that one of these plants was probably the food plant. Ball and Klingenberg (1935) collected adults "throughout the season" and suspected this was the hibernating stage. They found adults on many trees and shrubs and believed the species was a general feeder, or that the nymphal hosts were low, widespreading plants and adults flew to higher vegetation.

Although one species was reported to oviposit in *Yucca* (Townsend, 1892), Myers (1929) was the first to report the habitat of cixiid nymphs: *Mnemosyne cubana* Stål and *Bothriocera signoreti* Stål from Cuba. Instars of the *Mnemosyne* were found in a rotted stump in March. They had filamentous wax tails and were feeding on roots covered with this wax. Myers found the nymphs in apparent association with the ponerine ant, *Odontomachus haematoda insularis* var. *pallens* Wheeler. He stated that the ant was tending the nymphs, although he did not actually observe this activity. In addition, the ponerines are primitive ants; none are known to attend homopterans (Nixon, 1951). Late instar nymphs of *Bothriocera* were found under stones feeding on rootlets in March. The nymphs were covered with a white flocculent wax. In neither case, apparently, did Myers determine the identity of the food plants.

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The nymphs of the cixiid *Oliarus vicarius* (Walker) have been found feeding on roots beneath rotted stumps and logs in the same ecosystem as *O. borealis* (Thompson et al., 1979). Two species of ants seemed to be associated with these nymphs. The ant-Homoptera relationship reported in this paper is distinct, and appears to be obligatory for the *Oecleus* nymphs.

MATERIALS AND METHODS

The nest of *P. arenivaga* is marked by a distinctive crater. This ant is the predominant ant species in the sand pine habitats in which it is found. Excavations were undertaken in sand pine woods, *Pinus clausa* (Engelm.) Sarg. ca. 27 km east of Silver Springs, Florida (Marion County) in Ocala National Forest, on the south side of Highway 40. The vegetation in this area was composed of saw palmetto, *Serenoa repens* (Bartr.) Small, and turkey oak, *Quercus laevis* Walter.

After experimentation with various collecting methods, I found two effective in excavating maximum numbers of nymphs. One method was to shovel 4 vertical 20 cm thrusts to form a square around the crater of a *Paratrechina* nest. The entire clump was lifted and transported to a flat surface. It was broken apart with the hands while having an aspirator ready. The clump often broke, particularly when wet, along the tunnels of the ants. A second method, utilizing the element of surprise, was to thrust a shovel downward 3 to 4 cm from a crater and then carefully flip the shovel upwards. The nymphs were usually found among the greatest concentrations of ants. In this quick-attack method, the nymphs could be collected before they had time to escape. They were placed in separate vials from the ants.

Difficulties were encountered during warm weather in keeping the nymphs, ant queens and even ant workers alive until they were brought to the laboratory. It was found that moisture must be available at all times. To maintain collected live specimens in good condition, shell vials containing the insects were buried in a shaded place. Upon departure, tubs were filled with cool, moist sand, into which the vials were reburied. This method allowed successful transportation of live specimens during hot weather.

Captured *Oecleus* nymphs were brought to the laboratory and placed in Wilson cells (Wilson, 1962) with moist cotton floors. To approximate the *Oecleus* natural habitat, roots and sand from their original chambers were placed with the nymphs. The nymphs were kept under observation and disturbed as little as possible.

Radioisotype tracer studies and field observation were used to study food plant preferences of *Oecleus* nymphs. Prior to the isotope experiment, roots of three different plants were repeatedly found in the *Paratrechina* nests. Two of these root types were identified as pine and saw palmetto. Subsequently, young pine and saw palmetto plants were dug up and brought to the laboratory. Their roots were sandwiched between small pieces of non-absorbent cotton (to stop possible isotope leaks in the external root surfaces), then between small, back-to-back petri dish lids. The root space was taped shut around the sides of the petri dishes with water-proof tape, then packed tightly full of sand. The protruding roots were placed in water containing 100 μCi P^{32} per plant. After 24 hr, the nymphs were placed in cells through which passed roots in direct contact with the tracer solution. After contact with the plants for 36 hr, the nymphs were tested for radioactivity by both Geiger-Mueller and liquid-scintillation counters.

In a preliminary experiment, nymphs exposed to radioactive pine roots died after becoming trapped in water film which had collected on the inside chamber wall. To reduce film formation, the facing plastic of each chamber, sealed with clay against the petri dish, was perforated with a hot needle.

RESULTS AND DISCUSSION

Nymphs of *O. borealis* were discovered while excavating nests of the ant *P. arenivaga* in Ocala National Forest, Marion County, Florida. *Oecleus borealis* nymphs were also found in *P. arenivaga* nests in Putnam County, ca. 48 km north of the first discovery site, and later, in Broward and Orange Counties. These areas had similar habitats and were characterized by areas of white sand.

Many hundreds of nests were dug up during the course of this study. *Oecleus* nymphs were found in the *Paratrechina* nests from December through August (Table 1). Subsequent collections have been made in September and November (Wilson et al., 1983). The largest number of nymphs found in a single nest was five, but the actual numbers present could not be determined because of the methods used to excavate the nests. The ratios of nests with, to those without, *Oecleus* varied, but there was a pattern. During most months the ratio was 1:5, 6 or 7. In December–February the ratio was often 1:20 or more. At the other extreme, during July the ratio was reduced to 1:2.

Paratrechina nests were not found in heavily shaded areas. The preferred habitats of the ants were clearings, pathways within the woods, or disturbed areas including roadways. Nests were found only where the sand was clear of thick debris. A cover of sand pine needles was sufficient to exclude the ants from an area. Even when the nests were in the middle of a wide road, the roots of sand pine, saw palmetto, and what was probably turkey oak were found thickly matted beneath the soil surface. In some cases, measurement showed the nearest plant to be 3–4 m away.

Areas with similar vegetation, but without *Paratrechina* nests, were also excavated in attempts to locate *Oecleus* nymphs. These efforts were not successful. *Oecleus* nymphs were not found except in the company of the ants.

The nymphs collected varied in length from 0.8 mm to 3.7 mm. The smallest nymphs were faint yellow, darkening in later instars to bright yellow with orange bands on the abdominal segments. This coloration made the nymphs highly visible against the sand of the ant nests. Late instars had wing pads and dark reddish orange markings on the abdomen. The posterior white wax filaments were rarely found on the nymphs in the field, but grew out on nymphs in the laboratory within 12 hr. Full descriptions and a key to the nymphs are in Wilson et al., 1983.

Oecleus nymphs were repeatedly observed directly in the chambers of the ants. In at least 6 cases (2 in the laboratory, 4 in the field) ants were observed carrying first or second instar nymphs in their mandibles. This appeared to be a reaction to colony disturbance. When nests were dug between 9 or 10 A.M. and 4 P.M., few if any, nymphs were found. Temperatures are highest during this time and the sand becomes dry and hot in the upper layers. The nymphs and ants may avoid the heat by retreating down into the sand. During this time I was unable to find the ants or nymphs by digging downward ca. 30 cm. One nest was excavated to a depth of 80 cm and searched laterally, layer by layer, but no queens, brood or nymphs were found. The nymphs were also less frequently found if no rain had recently fallen. This was

Table 1. Collections of *Oecleus borealis* nymphs in *Paratrechina arenivaga* nests.

Date	Numbers of nymphs	Location	Notes
1/11/75	5	Ocala	—
1/25/75	4	Ocala	—
3/22/75	3	Ocala	2-L nymphs
4/5/75	4	Ocala	—
4/13/75	5	Putnam	1-S, 2-M, 2-L nymphs
4/21/75	1	Putnam	1-S nymph in worker's mandibles
6/18/75	9	Ocala	8-S, 2-M, 2-L nymphs
6/19/75	13	Ocala	—
6/29/75	2	Ocala	—
7/16/75	18	Ocala	—
7/25/75	1	Ocala	—
8/2/75	10	Ocala	—
8/4/75	3	Gainesville	1-M nymph
8/12/75	0	Ocala	—
12/—/75	1	Ocala	1-M nymph
1/22/76	0	Ocala	—
2/19/76	1	Ocala	1-M nymph
3/22/76	1	Ocala	1-S nymph
3/31/76	8	Ocala	5-S, 3-M nymphs
4/1/76	2	Ocala	2-L nymphs
4/5/76	2	Ocala	3-L nymphs
4/6/76	1	Ocala	—
4/15/76	1	Ocala	1-L nymph
4/27/76	8	Ocala	2-S, 6-M nymphs
5/10/76	6	Ocala	2-L nymphs
7/2/76	3	Putnam	1-M, 2-L nymphs
7/15/76	24	Putnam	24-S nymphs

Explanation of letters: S, M, L = estimates of *Oecleus* nymph size (Small, Medium, Large).

probably due in part to the tendency of the dry sand blocks to fall apart during excavation.

During collecting trips in December and January, the gasters of *Paratrechina* workers dug up were extremely distended with a clear liquid. This coincided with the presence of relatively large *Oecleus* nymphs in the ant nests. It is known that 1 aphid can produce copious amounts of honey dew relative to its size and weight and that the honey dew of some aphids is high in amino acids, protein and other nutritional substances (Way, 1963). If the *Paratrechina* are obtaining honey dew from the *Oecleus,* a relatively small number of nymphs might be able to support an entire colony—or at least to provide a substantial amount of its food supply.

Observations of *Paratrechina* behavior support this hypothesis. *Paratrechina arenivaga* is a crepuscular and mostly nocturnal ant and excavates or repairs its nests at that time. Many nights I have observed the ants, at the moment the head lamp light hit them, doing no foraging; carrying only sand grains. The light caused them to retreat: the workers quickly dropped the sand on the crater and returned down the entrance. When offered baits of honey, sugar water, corn syrup, egg or raw hamburger, the ants were most attracted to the sweet solutions, but consumed all the baits. Aside from the bait experiment, however, *Paratrechina* workers were not observed returning to a nest with anything in their mandibles or with distended gasters. They were rarely found more than 20 cm from their nest entrances. It is possible that these ants obtain their food from an underground source, perhaps the cixiid nymphs, at least during the winter months.

Attempts to rear *Oecleus* nymphs were not successful until several large nymphs were collected in March 1975. These were placed in Wilson cells with *Paratrechina* workers. An adult emerged several days later, but was almost entirely eaten by the ants. The ants were removed and a second adult emerged. It was found in an arm of the Wilson cell. The exuvia was clinging by the tarsi to the inside cell wall. This adult was determined to be *Oecleus borealis* Van Duzee.

In the pine and saw palmetto experiment, the radiation levels of roots removed only from the nymphal chambers varied from 113 to 2,585 cpm on the Geiger-Mueller counter and from 637 to 21,450 cpm on the liquid scintillation counter. Every set of roots had a count well above background on even the less sensitive Geiger-Mueller counter. None of the 5 nymphs recovered were radioactive.

A number of factors may have caused the non-radioactivity of the nymphs. Some of these are: 1) the plants were too young, possessing roots too small, or lacking sufficient sap; 2) sap flow in the plants was affected by the methods used to handle the plants or roots; 3) the nymphs were not able to feed due to handling methods or unnatural surroundings; or 4) neither of these plants is the natural food plant of *O. borealis* nymphs.

The difficulty involved in ascertaining the food plant of the *Oecleus* nymphs in the field lies with tearing apart the soil of the nest without disturbing these sensitive insects. Many times a nest was dug to find the nymphs already disturbed and jumping about (some can jump at least 8 cm). Other subterranean Homoptera known to associate with ants, such as coccids and aphids, are relatively sessile organisms. Even when alarmed, these insects are not capable of jumping and running, while this is the usual reaction of an *Oecleus* nymph. Even if the nymphs feed on ant nest roots and the ants are obtaining honey dew, it is doubtful that the ants could tend large nymphs directly. This is because the nymphs appear to avoid the ants (see below) and can move much more quickly than the ants themselves.

A number of interesting behaviors were noted in laboratory Wilson cell groups of nymphs and ants. The nymphs would not remain with the ants. If antennated by the ants, the nymphs moved quickly away. If antennated a number of times within a short period, the nymphs jumped away from the ants. The nymphs rested on roots in the cell, but did not seem to select one kind of root over another to rest upon. One nymph on a root was observed to move around the root and, in a squirrel-like motion, kept the root between itself and a nearby ant. The nymphs were extremely sensitive to any jarring of the cell, which caused them to move around or to jump.

During a 24 hr observation period, the wax filaments of a nymph's tail slowly lengthened ca. 2 mm. At one point, the nymph jumped, and when it landed the tail filaments had been flicked off. The most unusual behavior noted for a nymph occurred at 7:52 A.M., 21 hr after observation had begun. A medium-sized nymph was observed arching its head and abdomen downward, bending itself into a C shape. These movements continued periodically until 9:37 A.M., when the abdominal intersegmental membranes began to protrude from between the original abdominal plates. By 10:11 A.M., three new abdominal bands as wide as the 4 abdominal plates were present. They were a slightly lighter yellow-orange and glistening. The nymph arched, often while clinging to a root, and the haemolymph was observed flowing up and down in the proboscis and head. A change occurred in the nymph's behavior. Although it still avoided the ants, the nymph became extremely active. It had jumped only 3 times prior to 10:30 A.M. (twice in response to ant antennation), but between 10:30 and 11:00 A.M., when observation was terminated, it jumped 15 times. The nymph also continued to arch periodically during this time.

CONCLUSIONS

The nymphs of the planthopper *O. borealis* were found in the subterranean nests of the ant, *P. arenivaga.* These nymphs, members of the homopteran family Cixiidae, were previously unknown. Adding to Wilson's (1971) catalog of associations, Cixiidae is the ninth homopteran family known to be symbiotic with ants. Nymphs were present in the ant nests throughout the year.

It is suggested that the ant–nymph relationship is obligatory for *Oecleus,* involving the need for *Oecleus* to reach its host plant roots, which it does by penetrating a *Paratrechina* nest where roots are exposed by excavations of the ants. In all known ant–homopteran relationships, the ant obtains honey dew from the homopteran. In the ant–*Oecleus* relationship, this is also presumed to occur, but was not observed in field or laboratory colonies.

Although attempts to determine the *Oecleus* food plant with P^{32} were not successful, the author believes the technique was valid with the exception of nymph handling. Better handling techniques are needed since many nymphs succumbed to or did not feed under experimental conditions.

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